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MISCELLANEOUS PUBLICATION 3 IMMUNOFLUORESCENCE,

V. DIAGNOSTIC APPLICATIONS

AND REVIEW ARTICLES

Warren R. Sanborn

CLEARINGHOUSE FOR FEDERAL SCIENTIFIC AND TECHNICAL INFORMATION Hardcopy Microfiche

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UNITED STATES ARMY BIOLOGICAL LABORATORIES FORT DETRICK

U.S. ARMY BIOLOGICAL LABORATORIES Fort Detrick, Frederick, Maryland

MISCELLANEOUS PUBLICATION 3

IMMUNOFLUORESCENCE, AN ANNOIATED BIBLIOGRAPHY

V. DIAGNOSTIC APPLICATIONS AND REVIEW ARTICLES

Warren R. Sanborn

December 1965

Pathology Division
DIRECTORATE OF MEDICAL RESEARCH

FOREWORD

The use of immunofluorescence, or fluorescent antibodies, was initiated by Dr. Albert H. Coons and his co-workers in 1942. Dr. Coons has modestly stated that making antibodies fluorescent was "simply another variation of their use as reagents for the identification of specific antigen. . . ." However, this "variation" has proved to be one of immense significance to modern immunology. Its importance lies in the wedding of the two broad areas of investigation, morphology and immunology, thus allowing the detection of immunologic reactions at the cellular level.

The expanding volume of literature covering uses of immunofluorescence bears witness to the value of the technique. Through 1954 only about 40 artic es had been published. In the next two years 58 articles were published. In 1957 and 1958 there were 83 and 96, respectively. By 1961 this figure had risen to more than 260 in that one year alone. Apparently more than 400 articles per year can be expected for 1964 and 1965.

It would be virtually impossible to cite every article that refers to the use of immunofluorescence, but an attempt has been made in this six-volume annotated bibliography. Fifteen languages are represented, and more than 150 journals have been searched. Six abstracting journals have been included in the search. Translations were provided by several co-workers, government translating services, and the compiler. The earliest entry is dated 1905; significant publications through 1962 are included. Subsequent entries are being compiled and will be incorporated into revisions of this bibliography. The additions will, no doubt, increase considerably the bulk of these volumes.

The bibliography is intended to aid investigators in following the expanding mass of literature on the technique and to improve their skill in its use. The entire publication, Miscellaneous Publication 3, carries the title: "Immunofluorescence, an Annotated Bibliography." The subtitles for the six volumes are: Volume I, "Bacterial Studies"; Volume II, "Viral Studies"; Volume III, "Studies of Fungi, Metazoa, Protozoa, and Rickettsiae"; Volume IV, "Studies of Animal Physiology"; Volume V, "Diagnostic Applications and Review Articles"; and Volume VI, "Technical Procedures." Each of the volumes is subdivided into subject categories that should, hopefully, aid the reader in finding pertinent information in his field of interest without his spending undue time in scanning superfluous citations. Articles within subject categories are arranged alphabetically by senior author.

Accession numbers in each volume were assigned to articles by tens to allow room for expansion in subsequent editions. Accession numbers within each volume are consecutive throughout that volume, so the volume number must accompany the accession number to identify an entry unmistakably. Entries applicable to more than one subject category appear more than once, and these will have an accession number for each placement in the volumes.

A complete author index is included in each volume; the author's name is listed with the accession numbers of the entries with which he is associated. The asterisk designates those for which he is senior author.

The second parts of Volumes V and VI contain only references to articles printed in the other four volumes. As in the other volumes, the references are placed in subject categories, and are arranged alphabetically by senior author within categories. The authors, the year of publication, and the volume and accession number are shown to indicate where the complete entry can be found.

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For brevity, certain abbreviations in common usage in this field have been used rather than the more ponderous longer form. For unmistakable identification, they are listed below.

DANS 1-dimethylaminonaphthalene-5-sulfonic acid 5-dimethylamino-l-naphthalene sulfonic acid or its sulfonyl chloride form. FIC fluorescein isocyanate FITC fluorescein isothiocyanate FTA fluorescent treponemal antibody FTA-200 a modification of the above based on serum dilution. primary atypical pneumonis, PAP PAS para-aminosalicylic acid phosphate-buffered saline PBS a. lissamine rhodamine RB 200 RB 200 b. lissamine rhodamine B 200 c. lissamine rhodamine B đ. sulphorhodamine B acid rhodamine B e. Treponema pallidum fluorescent antibody **TPFA** TPI Treponema pallidum immobilization

Generally, the citations follow the format prescribed by the second edition of "Style Manual for Biological Journals," American Institute of Biological Sciences, 2000 P Street, N.W., Washington, D.C., 20036. Abbreviations follow "American Standard for Periodical Title Abbreviations, Z39.5-1963, American Standards Association Incorporated, New York.

The compiler started collecting this information in 1959 while he was stationed at the U.S. Army Biological Laboratories, Fort Detrick, Frederick, Maryland. Since his transfer to the Naval Medical Research Institute, Bethesda, Maryland, in 1963, he has continued this work with the encouragement and support of both installations.

The information in these volumes was originally recorded on coded Keysort cards. With the compilation of this publication, the citations and annotations have been transcribed on punched tape for conversion to automatic data processing and for use in updating later editions. Each entry is coded for recall by authors, date, title, and source publication to allow compilation of more selective listings.

Readers are invited to report errors or suggest added entries to the compiler or to Editorial Branch, Technical Information Division, U.S. Army Biological Laboratories, Frederick, Maryland, 21701, for improvement of the subsequent editions.

ACKNOWLEDGMENTS

This compilation would have been much more difficult if not impossible without the guidance, help, and encouragement of:

Dr. Harold W. Batchelor, who introduced the compiler to card-sorting systems;

The staff of Naval Medical Research Institute, who provided both support and personnel for assistance in this work;

My colleagues at the U.S. Army Biological Laboratories and the Walter Reed Army Unit at Fort Detrick and at the Naval Medical Research Institute in Bethesda, who volunteered their technical competence and supplied moral support.

Obviously, outstanding cooperation and assistance of librarians was required for this work. The staff of the Technical Library, Fort Detrick, under the direction of Mr. Charles N. Bebee, was continually patient, understanding, and essential for the entire period of compilation.

Another essential in the chain to final publication: Mrs. Madeline D. Warnock and her staff in the Editorial Branch at Fort Detrick have served as editors, of course, but also as confessors, encouragers, consciences, and the required driving force, all of which have brought this to its publication. The compiler is most grateful to Mrs. Warnock and her people.

ABSTRACT

This volume is one of a series of six annotated bibliographies on various aspects of immunofluorescence and its use. Citations cover the period 1905 through 1962 Volume V is divided into two major sections. The first section contains 85 annotated citations to review articles on immunofluorescence arranged according to major subject areas. The second section is devoted to diagnostic techniques. It contains 221 cross-references to citations in the other volumes of this series, arranged to correspond with subject matter areas in those volumes. A complete author index to the 306 citations is included.

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I. REVIEWS

A. GENERAL REVIEWS

10

Anonymous. 1961. Fluorescent protein and antibody tracing. Can. Med. Ass. J. 85: 1158-1160.

The various applications of the fluorescent antibody technique are generally presented. Possibilities of application in other biological sciences are pointed out.

20

Beutner, E.H. 1961. Immunofluorescent staining: The fluorescent antibody method. Bacterial Rev. 25:49-76.

This is a general review of the fluorescent antibody teamique. Topics covered include principles of immunofluorescent staining, antibody synthesis and reaction in vivo, immunofluorescent staining of microorganisms, immunofluorescent staining of tissue antigens.

30

Beutner, E.H. 1961. Ultraviolet light microscopy and the fluorescent antibody method. N. Y. State J. Med. 61:444-454.

In research, observations made with a microscope should be related to other types of observations. In ultraviolet light microspectrophotometry, observations are related to the absorption spectra of biologic substances. Notable contributions have been made to our understanding of the structure and behavior of cells by this method. In studies of secondary fluorescence, fluorochroming reactions have been related to the presence of acid-fast bacilli, RNA, DNA, and other factors. Microscopic observations are as meaningful as the observations to which they can be related. The fluorescent antibody method is among the most meaningful forms of microscopy because it relates staining reactions to the reactions of antibodies. Observations made by this method have contributed to our fund of basic understanding of antibody formation, virus infection, autoimmune reactions, and other problems of basic medical importance. However, to obtain meaningful results this method must be controlled as carefully as any other serologic method.

40

Borek, F. 1961. The fluorescent antibody method in medical and biological research. Bull. WHO 24:249-256.

This article is a general review of the history, background, applications, theory, and advanced technique developments of the fluorescent antibody procedure. A general discussion of various fluorochromes, their advantages, disadvantages, and chemistry, is presented. A general discussion and comparison of over-all advantages and disadvantages of the technique is included.

Borel, L.J.; Durel, P. 1960. Immunofluorescence, its possibilities in parasitology. Pathol. Biol. 8:65-68. In French.

Following a brief resume of the history of the fluorescent antibody technique, a discussion of the materials required is presented along with the methods for reagent preparation. The author points out the general possibilities of the method and the fact that its specificity is a function of the purity of the reagents. The hazards of and controls against nonspecific fluorescent reactions are pointed out. The article summarizes some of the parasitological applications of the method through 1958.

60

Cioffi, L.A. 1958. Fluorescent antibodies. Diagnosi 14:93-118. In Italian.

This is a review of the fluorescent antibody technique, including practical applications of this histochemical tool.

70

Coons, A.H. 1951. Fluorescent antibodies as histochemical tools. Federation Proc. 10:558-559.

The antibody molecule can be conjugated with fluorescent compounds without apparent loss of specificity. This fact makes it possible to employ the precipitin reaction as a method for the histochemical detection of small quantities of material in tissue cells. The only basic requirement for success is that the material sought be sufficiently antigenic so that the requisite antiserum can be produced.

80

Coons, A.H. 1954. Labelled antigens and antibodies, p. 333-352. In C.F. Clifton, ed. Annual reviews of microbiology, Vol. 8. Annual Reviews, Inc., Palo Alto, Calif.

Problems that have been studied by means of labelled antigens and antibodies include the cellular distribution of injected antigenic substances, their persistence in the blood and tissues, the effect of antibody on their persistence, the nature of the degradation products, and, by inference, the sites of antibody formation and its mechanism. For comparison, a limited number of studies have been carried out on similar nonantigenic molecules, i.e., homologous proteins.

90

Coons, A.H. 1956. Histochemistry with labelled antibody. Bull. N. Y. Acad. Med. 32:168.

Antibodies labelled with approximately two molecules of fluorescein per protein molecule can be used as specific fluorochrones for the localization of antigenic material in tissue cells. After applying a labelled antibody solution to a tissue section for long enough to allow the labelled antibody to be bound by the antigen present, then washing off the excess, the fluorescent protein deposit can be visualized under the

fluorescence microscope. Fecent modifications in this method by means of layers have allowed the demonstration of antibody in the cells of immune animals. When sections of lymphoid tissue from such animals are exposed to a dilute solution of the specific antigen, then antigen is bound by the antibody in the tissue cells. If excess antigen is washed away, the deposited antigen can then be detected by specific labelled antiserum, and hence the antibody present in the tissue cells can be visualized. Another use of layers has also been developed. When unlabelled specific antiserum is placed over a tissue section containing antigen, the globulin molecules are deposited from the serum over the sites of antigen localization. After excess unbound serum has been washed away, these globulin sites can be stained with specific fluorescent antiglobulin serum.

100

Cooms, A.H. 1956. Histochemistry with labelled antibody. Intern. Pev. Cytol. 5:1-23.

The use of antibody coupled with a visible latel has allowed the microscopic study of cells for their content of complex biological substances, and potentially it has placed a large body of immunological knowledge at the service of the histochemist. Thus far, however, it has been exploited principally for immunological ends. Eeginnings have been made in the investigation of antigens normally present in cells. The principal problem facing the investigator who wishes to use these immunohistochemical procedures in the study of normal tissue components will be the purification of the chosen antigenic material, in order to stimulate the synthesis of the necessary antibody in some other convenient species. Unexpected reactions are inevitable as small amounts of active antigens contaminating the material stimulate a disproportionate amount of antibody. The antigenic complexity of the erythrocyte is a strong hint as to the complexity of other cell types.

110

Coons, A.H. 1958. Fluorescent antibody methods, p. 399-422. In General cytochemical methods, I. Academic Press, New York.

This chapter is a basic reference for the fluorescent antibody technique. Easic theory is presented, followed by detailed instructions for all phases of the method. Instrumentation and tissue preparation are discussed, as are methods of entiserum preparation, conjugation, conjugate purification, tissue preparation, and staining. Problems of the present and future of this technique are a topic, plus review of some accomplishments using fluorescent antibodies through 1956. Appendixes contain additional technical information.

120

Coons, A.H. 1959. Antibodies and antigens labeled by fluorescein. Schweiz. Z. Allg. Pathol. Bakteriol. 22:693-699.

Antibodies labeled with fluorescence are coming into increasing use for localization of various kinds of antigenic molecules in tissue. The importance of large molecules in the structure and function of living things and the high degree of specificity of

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Coons, A.H. 1959. Antibodies and antigens labeled by fluorescein. Schweiz. Z. Allg. Pathol. Bakteriol. 22:693-699.

Antibodies labeled with fluorescence are coming into increasing use for localization of various kinds of antigenic molecules in tissue. The importance of large molecules in the structure and function of living things and the high degree of specificity of

immune reactions make it potentially useful in unravelling many biological problems, whether they are concerned with infectious disease, normal cell structure, or normal synthetic processes. It is a morphological method from which morphological answers are obtained, and will find its best if not its only use in such a context. Although it is possible to measure the light emitted from areas of fluorescence, quantitation of immune fluorescence reactions will probably be an enterprise of great technical difficulty. The determination of the presence and localization of a given antigenic substance will obviously be no more precise than the purity of the antibody solution allows. Although the chemistry of labeled proteins and the morphological form into which the results of such a method are placed can attract workers from scattered disciplines, the basis of the method lies in the immunological reaction, and in the care with which the immunological girders have been designed and placed.

130

Coons, A.H. 1960. Immunofluorescence. Public Health Rep. 75:937-943.

Some of the uses to which the fluorescent antibody technique has been applied are summarized. The importance of careful analysis of the antibody being used is stressed.

140

Coons, A.H. 1961. The beginnings of immunofluorescence. J. Immunol. 87:499-503.

Dr. Coons sketches the beginnings of his work with fluorescent antibody. He became interested in immunology while a medical student and in 1935 began work with John Enders at Harvard. With the help of Fieser, Creech, Jones, and Berliner, he produced fluorescein isocyanate and in 1° reported an antipheumococcal conjugate.

150

Darken, M.A. 1961. Natural and induced fluorescence in microscopic organisms. Appl. Microbiol. 9:354-360.

A review.

160

Darken, M.A. 1961. Applications of fluorescent brighteners in biological techniques. Science 133:1704-1705.

The visual labeling of various microorganisms has been accomplished with specific fluorescent ultraviolet-absorbing compounds that not only have been bound by the coll but have been transferred by it to subsequent growth. The possibility of application to genetic studies is suggested.

deRepentigny, J. 1958. Some limitations in the use of the fluorescent antibody technique. Rev. Can. Biol. 17:492-502.

Experimental work has shown that chromatography can be used to simplify the separation and analysis of aminofluorescein isomers; that the fluorescent antibody technique can be applied to the study of the antigenic structure of bacteria mainly surface antigens; and that the autofluorescence present in many bacteria, needs to be determined in the controls with greater accuracy, especially when the color of the fluorechrome is fluorescein-like. Results point to the necessity of quantitative methods in the use of the fluorescent antibody technique. It is hoped that some kind of standardization of the technique can be achieved in order that all investigations in this field may yield comparative results.

180

deRepentigny, J. 1961. The fluorescence microscope in microbiology. Trans. Royal Soc. Can. 55:5:5-14. In French.

These studies indicate that the fluorescence microscope can contribute much to microbiological research. Among its applications the fluorescent antibody method can be made more standard through determination of the natural fluorescence of microorganisms.

190

De Teyssie, A.R. 1961. Immunofluorescence and its application. Semana Medica Buenos Aires 119:1975-1977. In Spanish.

A review.

200

Eisenbrand, J.; Werth, G. 1959. Fluorescence microscopy. 2nd ed. Geest and Portig, Leipzig. 168 p.

This is a basic text on the subject, including fluorescent antibodies.

210

Fagraeus, A. 1958. Cellular reaction in antibody formation. Acta Haematol. 20:1-18.

This review article includes fluorescent antibody among the methods considered to be the most specific techniques for this type of study.

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Faivre, G.; Gilgenkrantz, J.M.; Debever, J. 1962. Fluorescent antibody technique applied to the study of post-commissurotomie syndrome. Sem. Hosp. Paris 38:2307-2310. In French.

This is a general review article with some specific reference to myocarditis and post-infarct immunopathology.

230

Ginodman, L.M. 1957. The use of fluorescent antibodies, its present status. Probl. Virol. 2:4:202-208.

This general review article follows protein tracing from Russian work on tetanus towin, through radioactive methods, to the Coons technique. Chemistry of the problems involved is briefly discussed.

240

Haurowitz, F. 1960. Immunochemistry. Annu. Rev. Biochem. 29:609-634.

This is a general review of immunochemistry including a brief description of the fluorescent antibody technique and some of its applications.

250

Mellors, R.C. 1959. Fluorescent antibody method, p. 1-67. In R.C. Mellors, ed. Analytical cytology, 2nd Ed. The Blakiston Division, McGraw-Hill Book Company, Kew York.

In this chapter a general review of the background, techniques, materials, and uses of the fluorescent antibody technique is given. The introduction traces the history of development of the technique, followed by a graphically illustrated discussion of the general procedures and principles involved. Detailed instructions are given for the following items: Preparation of antisera, globulin fractions, fluorescein amine, fluorescein isocyanate- and isothiocyanate-labeled antibodies, FA properties, tissue sections, cytologic materials, and staining. Also included is a discussion of physical equipment available and the pertinent specifications. Recommended filters, their positions, and uses are pointed out. Microscopes and attachments are included, with a section on corollary microscopic methods. A resume of various uses of FA is given. The text is supplemented with tables and photographs.

260

Mikhailov, I.F.; Li Li. 1960. On the variants of the fluorescent sera method. J. Microbiol. Epidemiol. Immunobiol. 31:3:392-398.

This article includes a general background of the method and a short review of the part played by Soviet industry in preparing reagents and manufacturing equipment.

Considerable discussion is accorded the difficulty of the use of fluorescein isocyanate because of the complexity of preparation and problems of storage. Two methods, sealed acetone solution and desiccation, are discussed and experiments using these preparations are described. Freeze-drying was also successfully attempted. A review is given of staining techniques with emphasis on the indirect method.

270

Nairn, R.C. 1961. Fluorescent protein tracing and the fluorescent antibody method. Endeavour 20:78-84.

Fluorescent dyes may be used for labeling proteins without materially affecting their biological or immunological properties. This is most valuable for biological research, for such labeled proteins can be injected into animals and traced directly in histological sections by ultraviolet fluorescence microscopy. Alternatively, in immunological tracing, labeled serum antibody is used as a specific histochemical stain to locate the corresponding antigen in microscopical preparations. This article describes and illustrates the study in this way of a wide variety of antigens, including microorganisms and protein constituents of tissue.

280

Naumann, H.H. 1957. Fluorescence microscopic studies of tonsillar function: V. Reticuloendothelial system, plasma cells, lymphocytes, and antibody formation. Z. Laryngol. Rhinol. Otol. 36:4:195-214. In German.

This review article deals generally with primary fluorescence of tissues. Fluorescent antibody conjugates are mentioned.

290

Ornstein, L.; Mounter, W.; Davis, B.J.; Tamura, R. 1957. New horizons in fluorescence microscopy. J. Mt. Sinai Hosp., N.Y. 24:1066-1078.

Experiments and observations in fluorescence microscopy and general fluorescence principles are discussed. Reference is made to the work of Coons and the fact that isocyanates react with NH2, SH, and phenolic OH groups. Other topics discussed include sensitivity of absorption and fluorescence techniques, filter systems, light sources, and appropriate fluorescent dyes. Sources of errors and reduction of autofluorescence are included. Future possibilities for fluorescence microscopy are suggested.

300

Peltier, A.; Burtin, P. 1961. Preparation and utilization of fluorescent antibodies. Rev. Franc. Etudes Clin. Biol. 6:610-612. In French.

Various techniques for the preparation and use of fluorescent antibodies are described.

Poetschke, G.; Uehleke, H.; Killisch, L. 1957. Investigations with fluorescein-labeled antibodies: I. General methods. Z. Immunol. Exp. Therapie 114:4:393-405. In German.

The use of FA as a serological or histochemical procedure is described in review and in experiences of the authors.

320

Richards, O.W. 1955. Fluorescence microscopy, p. 5-1 to 5-37. In R.C. Mellors, ed. Analytical cytology, 1st Ed. The Blakiston Division, McGraw-Hill Book Company, New York.

Basic principles of fluorescence microscopy are presented. Details on the use of filters, including graphic curves, are particularly useful.

330

Shevliagin, V.Ya. 1958. Detection of antigens using fluorescent antibody. Usp. Sovrem. Biol. 45:2:218-233. In Russian.

This is a historical review.

340

Spink, W.W. 1962. The young investigator and his fluorescent antibody. J. Amer. Med. Ass. 181:889-891.

The author has used the example of the development of the fluorescent antibody method by Dr. A. H. Coons to illustrate the problems and successful techniques for guiding young scientific investigators. Philosophy of teaching and the problem of guidance versus allowance for development of conceptual ideas is discussed. Developments resulting from the work of Dr. Coons are briefly reviewed.

350

Sylvana Chemical Company. 1957. Fluorescent antibody method: Brochure. Sylvana Chemical Company, 22 East Willow Street, Millburn, N.J.

The basic principles of the technique are discussed. The basic consideration of the direct and indirect methods is outlined and pictorially represented. Various fluorescent microscope systems are noted.

360

Tanaka, N. 1958. Fluorescein-labeled antibody as a histochemical tool: Antigenantibody reaction on tissue sections. Seitai No Kaguku 9:215-225. In Japanese.

A description of fluorescent antibody staining of tissue sections is given.

White, J.D. 1959. Workshop on fluorescent antibody methods. Committee on Continuing Education, American Society of Pathologists. Pamphlet, 9 p.

This publication outlines the basic technique involved in fluorescent antibody use and gives directions for the conjugation procedure, staining procedure, and antiserum fractionation. The theory of controls in the test is presented with examples. Sources of microscopy equipment and fluorescent dyes are listed, along with basic reagent formulae.

380

White, R.G. 1960. Fluorescent antibody techniques, p. 89-104. In Tools of biological research, Vol. II. Charles C. Thomas, Springfield, Ill.

This is a review of the FA technique including its applications in histochemistry.

B. APPLICABLE TO DIAGNOSIS

390

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Anderson, R.J. 1958. Fluorescent antibody techniques. Publ. Health Rep. 73:884.

Fluorescent antibody techniques for rapid laboratory identification of pathogens, and of antibodies produced in man by these microorganisms, are under development at the Communicable Disease Center of the Public Health Service. These new diagnostic methods, which use a fluorescein dye to light up individual disease organisms, promise one day to enable the physician to make an accurate diagnosis of certain communicable diseases within minutes after the patient comes to his office. There follows a brief general description of the methods. Before general use, several practical problems will have to be solved. UV microscopes will have to be available and technicians trained to use the method. Tight budgets may delay use of the FA technique in some labs.

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400

Anonymous. 1958. Antibody-antigen reactions observed by fluorescence. Lancet 1:1166.

A brief review of the use of the fluorescent antibody technique in the diagnosis of infectious diseases is presented. Globulin affinities and their use in tumor detection are mentioned. Some of the problems encountered with the technique, especially in examining a very heterologous material such as feces, are discussed, and possible future uses of the method are pointed out.

410

Bedarida, G. 1961. Fluorescent antibody rethod for the diagnosis of infectious and autoimmune diseases. Riv. Emoterap. Immunoematol. 8:6:337-370. In Italian.

A review.

420

Bedarida, G.; Bernasconi, C. 1961. Immunofluorescence in the diagnosis of immunological thrombocytopenic disease: Technical problems and proposed new methods. Riv. Emoterap. Immunoematol. 8:6:307-316. In Italian.

This is a review of methods.

430

Blundell, G.P. 1961. General remarks on the status of fluorescent antibody technics as a diagnostic serologic test. Amer. J. Clin. Pathol. 35:255.

The advantages of the use of the fluorescent antibody method as a standard serological test for certain problems in the clinical laboratory are discussed. Advantages noted are rapid diagnosis, lack of culture requirements, and observation of organisms in the infected tissue.

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Blundell, G.P. 1961. Fluorescent antibody techniques in the pathology laboratory. Conn. Med. 25:413-416.

Fluorescent antibody is a useful research technique that has been successfully applied for identification of many antigenic substances. It currently may be reliably employed in a hospital laboratory, with commercially available reagents, for the diagnosis of syphilis, infant diarrhea due to E. coli 0127:B8, Group A streptococci, and rabies. The technique must be applied with care and with proper controls. Experience is required in the recognition of the yellow color of the fluorescein staining and in the evaluation of the degree of its intensity.

450

Bossi, G. 1962. The immunofluorescent test in the diagnosis of infection. Minerva Med. 53:95:3561-3566. In Italian.

A review.

460

Cherry, W.B.; Goldman, M.; Carski, T.R.; Moody, M.D. 1960. Fluorescent antibody techniques in the diagnosis of communicable diseases, Pub. Health Serv. Publ. 729. U. S. Government Printing Office, Washington. 73 p.

A history of the development of immunochemical staining procedures, with special reference to applications in the diagnostic field, has been presented. Theoretical and practical considerations of fluorescent antibody techniques are discussed. The preparation of reagents employed in immunofluorescence studies was explored and their availability is cited. The types of fluorescent antibody tests that are used most commonly are discussed and their application to diagnostic problems in the field of microbiology is presented in some detail. Certain practical problems that arise in the application of these tests to the diagnosis of communicable diseases are mentioned.

470

Coons, A.H. 1959. The diagnostic application of fluorescent antibodies. Schweiz. Z. Allg. Pathol. Bakteriol. 22:700-725.

This article is a summarized presentation of general diagnostic applications of fluorescent antibody with a concept of the immunological advances over the past 70 years. The fluorescent antibody concept is a broad advance in the serological identification of microbiological species. Diagnosis by fluorescent antibody and its staining application for bacteria, viruses, fungi, rickettsiae, and animal parasites is discussed in general. Concepts of different authors pertaining to the subject are introduced.

D'antona, D.; Marmucci, E. 1961. Fluorescent antibodies in laboratory diagnosis, basis of methodology. Annu. Sclavo. 3:1:37-47. In Italian.

Useful information is given in this paper on the application of immunofluorescence in laboratory diagnosis with special respect to the responsible physical factors, immune reactions on which methods are grounded, and the proper microscopic conditions.

490

Dulong de Rosnay, C.; Boineau, J. 1962. A method of immunological investigation of general interest: Immunofluorescence. J. Med. Bordeaux 139:1453-1470.

This is a review of FA techniques and applications.

500

Kaplan, W.; Kaufman, L. 1961. The application of fluorescent antibody techniques to medical mycology - a review. Sabouraudia, J. Int. Soc. Human and Animal Mycol. 1:137-144.

The significant applications of the fluorescent antibody techniques in medical mycology are reviewed. The review covers problems and the progress made in the development of FA reagents for the detection of Cryptococcus neoformans, Candida albicans and other Candida spp., Sporotrichum schenckii, Histoplasma capsulatum, and Blastomyces dermatitidis. The progress made in the application of this technique to measurement of fungal antibodies in sera is described. The authors briefly discuss the principles of the FA procedure, some of the problems encountered in performing the tests, and the potentialities of the technique.

510

Lake, A. 1962. Tracking killer germs. Sat. Eve. Post 235:76, 78.

This article is written to acquaint the lay public with fluorescent antibody as it is used to diagnose certain infectious diseases. Uses discussed are diagnosis of epidemic diarrhea, strep throat, rabies, venereal disease, and brucellosis. Field use in various city and county health departments is indicated, as are future diagnostic possibilities.

520

Liu, C. 1960. The use of fluorescent antibody in the diagnosis and study of viral and rickettsial infections. Ergeb. Microbiol. Immunitatsforsch. 33:242-258.

This paper reviews results in studies on the usefulness of fluorescent antibody in various immunohistochemical problems in the diagnosis of several viral and rickettsial diseases. It discusses mumps, influenza, fowl plague, adenoviruses, primary atypical pneumonia, psittacosis, varicella and herpes zoster, vaccinia, Egypt 101 virus, poliovirus, Teschen disease virus, which is the virus encephalomyelitis of

swine, canine distemper, viruses that produce inclusion podies, such as herpes simpley comine distemper, canine hapatitis, and rabies, miscellaneous viruses, such as Newcastle disease virus, Shope papilloma virus measles, and similar formy agent, and rickettsiae. The fluorescent antibody technique has been proven very useful, especially when morphologial localization of antigens in tissues is in question. One of the great advantages of the technique is the precise localization of antigens at the cellular level. When used a conjunction with other virological techniques, the fluorescent antibody technique can lead to the development of rapid diagnostic methods.

530

Mikhailov, I.F. 1958. Possible uses of the method of fluorescent antisers. J. Microbiol. Epidemiol. Immunobiol. 29:8:1312-1319.

A general review of the development and uses of the fluorescent antibody technique is given, primarily through a review of the literature. Reference is occasionally made to apparently unpublished work by the author. The problem of nonspecific reaction is discussed, with suggestions toward its reduction or removal. The indirect staining technique is mentioned. Emphasis is placed on specific staining of microorganisms such as bacteria, rickettsiae, viruses, and protozoa, but some mention is made of tissue work and basic investigations into the nature and sites of antibody production.

540

Nairn, R.C. 1962. Fluorescent protein tracing. E. and S. Livingstone Ltd., Edinburgh and London, 280 p.

This excellent book is a very complete reference work on the fluorescent antibody method. The discussions of theory are outstanding. Directions for various procedures, as well as many applications, are discussed.

550

Pomales-Lebron, A. 1962. The importance of fluorescent antibody technique in medicine. Bol. Ass. Med. Puerto Rico 54:409-411. In Spanish.

This is an introductory article generally describing the method, equipment, and diagnostic applications. It is concluded that FA is an indispensable tool in the modern medical laboratory.

Ravaioli, L. 1961. Fluorescent antibodies and their practical applications. Zooprofilass 16:43-61. In Italian.

This work consists of a short review of the state of knowledge on fluorescent intibodies and their practical application. Descriptions of the materials and methods, antiserum preparation, conjugation, purification of conjugations, and fluorescent microscopy are given. Techniques of application are discussed.

570

Roa, P.V. 1961. Fluorescent antibody technique. Indian J. Med. Sci. 15:34-35.

The fluorescent antibody technique is described briefly. Its uses are indicated in the fields of virology, bacteriology, immunochemistry, and others.

580

Sinitskiy, A.A.; Diakov, S.I.; Osipova, I.V. 1959. Application of fluorescent antibodies to the detection of pathogenic microbes. Voenno Med. Zh. 4:35-40. In Russian.

FA is mentioned in reference to accelerated bacteriological diagnosis of infectious diseases. Various applications of the method including foreign protein tracing, homologous protein tracing, and study of pathogenic bacteria and viruses are discussed. Accelerated diagnosis and detection by means of fluorescent antibodies remains tentative, a signal method, and does not eliminate the need for carrying out classic methods of microbiological diagnosis.

590

Smith, C.W.; Metzger, J.F.; Hoggan, M.D. 1962. Immunofluorescence as applied to pathology. Amer. J. Clin. Pathol. 38:26-42.

The fluorescent antibody technique as applied to pathology is discussed, with the emphasis on the problems still present. Immune sera of higher degrees of specificity are needed. Applications of recently discovered biophysical techniques could provide a method of separating and specifically identifying antigenic components, thus eliminating undesirable cross-reactions. At the present time, identification of pathogens in tissue sections with fluorescent antibody is possible only in conjunction with clinical evaluation and observed pathologic conditions.

600

Sonnenwirth, A.C. 1962. Fluorescent antibody methods and their use in the diagnostic laboratory - a short review. Lab. Digest 26:14-16.

This is a review based on Fluorescent Antibody Techniques in the Diagnosis of Communicable Diseases, PHS Publication 729, 1960.

Stulberg, C.S. 1951. Immunofluorescence as a diagnostic tool. Amer. J. Dis. Child. 101:137-139.

Diagnostic FA applications are reviewed and discussed. FA has basic advantages over conventional procedures in speed, sensitivity, and specificity. Experience in use and interpretation of FA is required for satisfactory results, as with any diagnostic tool. Deterrents to use of FA, principally in preparation and storage of reagents and in obtaining suitable equipment, have now been largely overcome, and thus this technique is available to most diagnostic laboratories. FA is opening a new era in the diagnosis and control of infectious diseases.

620

Suzuki, S.; Furukawa, N. 1962. The application of fluorescent antibody technique in the field of pediatrics. Shonika 3:327-333. In Japanese.

This review article discusses the various FA techniques - direct, indirect, and complement staining. It also describes how to prepare specimens, staining methods, and equipment for fluorescence microscopy. Clinical applications of FA technique and diagnosis are considered in influenza, streptococcal infection, pertussis, diphtheria, and viral infections.

630

Fararin, R.A.; Zavarzin, A.V. 1961. Theoretical scientific conference on the problem of reducing and eliminating epidemic disease. VoennoMed. Zh. 12:129-131.

This is a report of a conference on the theoretical elimination of epidemic diseases in the armed forces. Some of the diseases studied were influenza, hepatitis, and dysentery. The need for joint cooperation of commanders, troops, and medical personnel is emphasized. A.V. Ponomarev, Colonel of the Medical Services, pointed out the need for modern microbiological techniques in diagnosis, and he noted the first of several tests in the use of the fluorescent antibody method. He emphasized the need for incorporation of fluorescent antibody into practice, especially with reference to virus diseases.

640

Pozzini, F. 1958. Fluorescent antibody technic in laboratory diagnosis. Zooprofilassi 13:560-563. In Italian.

This article outlines the basic method and reviews certain specific applications in the fields of pacteriology and virology.

C. APPLICABLE TO PHYSIOLOGY

650

Aubuchon, M. 1959. Labeling antibodies. Hospital Progress 40:122-123.

The use of the fluorescent antibody technique and its development through the years is reported. Recent work to prove that the stimulation of antibody production in rheumatic fever cases causes possible heart damage is included.

660

Bedarida, G. 1962. Use of fluorescent antiglobulins in research on antileukocyte antibodies. Riv. Emoterap. Immunoematol. 9:2:95-111. In Italian.

Time is a review of methods. Nonspecific fluorescence reduction is discussed.

670

Chen, H.Y. 1962. Studies on the staining reaction of the fluorescent conjugate of globulin. Shih Yen Sheng Wu Hseuh Pao 7:283-298. In Chinese.

A review of past work is first presented. Then studies of the staining of sections of various tissues and tissue cultures are made with respect to staining intensity under varying conditions such as pH, ionic strength of diluents, fixation methods, and effects of normal versus tumorous tissues. An interesting point was the use of pH 12.5, which gave very intense staining and good differentiation between the nucleus and the cytoplasm of cells. The dyes DANS and RB 200 were used for conjugation.

680

Coons, A.H. 1957. The application of fluorescent antibodies to the study of naturally occurring antibodies. Ann. N. Y. Acad. Sci. 69:658-662.

This is the first instance of a clear cytological demonstration of an antigenic difference between normal and malignant cells. It does not establish the existence of a different antigen in tumor cells, but it implies a difference in their antigenicity, since, as they have lost a normal antigen, they have probably gained an abnormal one. The author indicates both the possibilities and the difficulties inherent in attempting to use immunohistochemical methods as an approach to the investigation of malignant tissue. Further details and bibliography are given in a review.

690

Engelhardt, G. 1958. The localization of antibody formation. Deut. Mel. Wochensch. 83:20:877-880. In German.

This is a broad review and comment on localization of antibody formation and the techniques used to study this phenomenon. As a portion of this paper, immunohistochemical studies are reviewed. Plasma cells were indicated as antibody-producing sites by this technique. Quantitation of antibody was not possible.

Faivre, G.; Gilgenkrantz, J.M. 1962. Value of immunofluorescence in cardiology. Actualites Cardiol. Int. 11:15-18. In French.

The theories for immunologic phenomena in cardiac diseases are reviewed and the contributions of FA by Kaplan are discussed.

710

Gluck, E. 1962. Fluorescent antibodies in cancer research: A review. Cancer Res. 22:895-897.

A review.

720

Herbeuval, R.; Herbeuval, H.; Duheille, J. 1962. Fluorescence and immunofluorescence in blood cytology. Strasbourg Med. 13:621-626. In French.

Applications of fluorescence microscopy and FA in study of white cell concentrations for evidence of cancer are discussed. The results of other workers and those of the authors are reviewed.

730

Seegal, B.C. 1959. The value of the technique using fluorescent antibodies in the study of experimental nephritis. Its possibilities for application to an interpretation of the pathogenesis of certain forms of human nephropathy. Rass. Fisiopathol. Clin. Terap. 31:12:1063-1078. In Italian.

This address is a review of work in experimental nephritis. The fluorescent antibody method is the principal study tool.

740

Seegal, B.C. 1962. The value of the fluorescent antibody technique in the study of chronic disease. J. Chron. Dis. 15:935-940.

This review article places emphasis on the chronic diseases of various etiologies. Included are discussions on microorganism-related disease, connective tissue disorders such as rheumatoid arthritis and lupus erythematosus, endocrinological problems as in Hashimoto disease, nerve demyelination mechanisms, and tumor factors such as viruses and abnormal antigens. Studies of myasthenia gravis, kidney disease, and rheumatic fever receive comment. Both diagnostic applications and mechanism of pathogenesis studies are discussed.

THE PARTY OF THE P

Wanler, E. 1962. The immunopathology of the collagen discuses. Acta Pathel. Microbiol. Scand. Suppl. 154:29-39.

This is a review of the collagen diseases: rheumatic fever, rheumatoid arthritic, systemic lupus erythematosus, diffuse scleroderma, dermatomyositis, polyarteritis nodosa, and thrombotic thrombocytopenic purpura. Principal discussion revolves around autoimmunity as demonstrated by FA.

D. APPLICABLE TO SPECIFIC ORGANISMS

760

Belyavin, G. 1959. New serological techniques in the field of virus research. Brit. Med. Bull. 15:193-196.

In this review the following methods are outlined and discussed: direct virus flocculation, virus tube flocculation, agar-gel diffusion precipitation, fluorescent antibody, haemadsorption, and immune adherence. In the section regarding fluorescent antibody the general technique is briefly outlined. A brief review of the significant results in virology using this method is given. The advantages of the fluorescent antibody technique over electron microscopy in specific identification are acknowledged.

770

Carski, T.R. 1961. The use and limitations of the fluorescent antibody technic in the identification and localization of viruses. Amer. J. Clin. Pathol. 35:260-262.

Fluorescent antibodies are used for the localization of viral antigens in tissues. Limitations of the method are stressed. Experiments must be carefully controlled to maintain the integrity of the antigen in the specimens; undesired staining reactions must be considered; and the techniques have yet to be proved reliable by extensive field evaluation.

780

Cherry, W.B. 1961. The use and limitations of the fluorescent antibody technic in the identification of bacteria in body fluids and exudates, and from cultures. Amer. J. Clin. Pathol. 35:256-257.

Fluorescent labelled antibodies are potentially applicable to the detection of all bacteria. The necessity of thorough investigation of each antigen-antibody system, however, cannot be overemphasized. Each must be evaluated for rapidity, sensitivity, and specificity of staining under the environmental conditions characteristic of the disease state. Known positive controls must be included at each step. Fluorescence tests and conventional procedures should be performed simultaneously on the same specimens until it is established beyond a doubt that the former are equal or superior to the latter. The diagnostic applications of the fluorescent antibodies in bacteriology are in the early stages of development, and at the present time no fluorescence test has supplanted the corresponding conventional procedure.

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Coons, A.H. 1956. The morphological aspects of virus infections of cells as revealed by fluorescent antibody. Ciba Foundation Symposium on the Nature of Viruses p. 203-207.

Antibodies labelled with fluorescein have been successfully employed to detect the antigens of a number of viruses inside the infected cell: mumps, influenza, infectious canine hepatitis, vaccinia, varicella, primary atypical pneumonia, Egypt (sic), canine distemper, measles, herpes simplex; psittacosis, and in preliminary experiments, poliomyelitis. It is likely, therefore, that cells infected with most viruses against which antibody can be obtained either by the immunization of animals, or from individuals convalescent from infection, can be visualized in this way.

608

Dashkevich, I.O.; Diakov, S.I.; Fermakov, N.V.; Ivanov, M.T.; Osipova, I.V. 1960. The use of the indirect fluorescent antibody technique for species-specific and type-specific staining of certain pathogenic bacteria. J. Microbiol. Epidemiol. Immunobiol. 31:2037-2043.

Fluorescent conjugates of chicken antirabbit gamma globulin labelled with fluorescein isocyanate were prepared that gave intense fluorescence and possessed high immunological specificity. A method for the species-specific and type-specific staining of certain pathogenic bacteria by the indirect fluorescent antibody technique was developed. It could be shown that in microbiological practice the indirect fluorescent antibody technique is superior to others, as it enables us to stain specifically various species of bacteria with only a single fluorescent antirabbit serum.

810

Glubokina, A.I.; Kabanova, Ye.A.; Levina, Ye.N.; Pishchurina, M.M. 1960. Technique of obtaining and applying, in microbiology, sera labelled with fluorescein isocyanate. J. Microbiol. Epidemiol. Immunobiol. 31:3:385-391.

This article is a review of the literature on the technique of use of fluorescent antibodies for staining bacteria. Detailed instructions are given for all aspects of serum handling, globulin precipitation, conjugation, elimination of nonspecific fluorescence, and staining of smears. Both the direct and indirect techniques with controls are described. Staining of B. anthracis and dysentery bacilli is specifically mentioned. The dye discussed is fluorescein isocyanate.

820

Mayor, H.D.; Melnick, J.L. 1961. Intracellular and extracellular reactions of viruses with vital dyes. Yale J. Biol. Med. 34:340-358.

A review of recent work related to the use of vital dyes in virus research. Included are studies on the photo-inactivation enhancing effect of dyes incorporated into developing poliovirus, the multiplication of poliovirus using correlated cytochemical

and fluorescent antibody techniques, and the use of acridine orange as a differential reagent for nucleic acids in purified viruses.

830

Meysel, M.N.; Kabanova, Ye.A.; Levina, Ye.N.; Pishchurina, M.M. 1957. Fluorescent antibodies and their application in cytology and microbiology. Izv. Akad. Nauk SSSR, Ser. Biol. 6:718-732. In Russian.

The fluorescent antibody method was deemed to be of great potential value to many medical fields. Techniques and their modifications for preparing reagents were investigated. Vaccinia virus and various bacteria were specifically stained. The authors felt that their technique alterations improved results of FA observations.

840

Poetschke, G. 1961. Demonstration of viruses and viral antigens with the aid of fluorescent-labeled antibodies. Progr. Med. Virol. 3:79-157. In German.

A review.

850

White, J.D. 1961. The use and limitations of the fluorescent antibody technic in the identification and localization of bacteria in specimens of tissue. Amer. J. Clin. Pathol. 35:257-260.

Problems and their solutions involved in the localization of bacterial antigens in tissue are discussed. Fixation methods for various types of antigens are suggested, along with the basic methods for tissue preparation. Autofluorescence and nonspecific fluorescence, and methods of overcoming these problems, are included, as is the importance of selecting appropriate controls.

II. REFERENCES TO DIAGNOSTIC APPLICATIONS

A. BACTERIA

1. Bacillaceae

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Cherry, W.B.; Freeman, E.M. 1959. See MP 3, Vol. I, No. 150.

870

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880

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2. Bedsonia (Miyagawanella)

890

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900

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3. Brucellaceae

930

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950

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4. Enterobacteriaceae

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1630

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2520

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2930

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2940

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Huebner, R.J. Hunter, D.K.	1640 1730*	Koeditz, H. Kotcher, E. Kozar, M.I. Kozhushko, M.I. Kravetz, H.M.	1160* 1020, 2700 1170 1170* 1610
Inhorn, S.L. Ishida, N. Ivanov, M.T. Ivanova, S.P. Ivanovsky, D.I. Ivens, S.	1450*, 1900* 2140* 800 1090 2410* 2460	Kunin, C.M. Kunz, C. Kusano, N. Kuznetsova, N.S.	2180 247C* 2190 1150
Iverson, L.	2490	LaBrec, E.H. Lake, A.	1180*, 2030 510*
James, W.D. Jenkins, M. Joel, W. Johnson, G.D. Johnson, K.M. Johnson, R.S. Johnson, R.T. Jordon, M.E.	1600 2330* 2070 2790, 2950, 3000 1610 1540, 1550 1640 2760	Larsh, H.W. Lawson, W.B. Lee, S. Leeuw, B. Lesko, M. Lessef, M.H. Levina, Ye.N. Lewis, A.L. Lewis, V. Leyton, G.R.	2440 1910 3010 2940 1310 2860, 3050* 810, 830 2340 2080 2300
Kabanova, Ye.A.	810, 830, 1140*, 1150*	Li Li Lindner, G. Lipkin, M.E. Liu, C.	260, 1200 1280, 1520 2040*, 2770* 520*, 1650*, 1660*,
Kalter, S.S. Kampelmacher, E.H. Kaplan, W. Kark, R.M. Kase, A. Kashiwazaki, H.	2230, 2250* 880* 500*, 2460* 2960, 2980 2490 2130, 2240	Lucasse, C. Lynch, H.J.	1670*, 2150*, 2160*, 2170*, 2180 2590* 2480*, 2510

<u>M</u>		. <u>o</u>	
Macaulay, J.C.	2120	Oikawa, K.	2190
Mandema, E.	2960*, 2980	Okuno, Y.	221.0
Mandras, A.	2710 *	Olansky, S.	1940*
Mannucci, E.	480	Ornstein, L.	290*
Marcheselli, W.	1800	Osipova, I.V.	580, 800, 1250*
Marcus, B.B.	2020	2000	, , , , , , , , , , , , , , , , , , ,
Margulis, I.L.	1170	<u>P</u>	
Marie, J.	980*	-	
Marshall, J.D.	2490*	Padula, J.F.	2520
Martin, M.M.	2180*	Page, R.H.	1000*, 1010*, 1100,
Martineau, B.	1190*	- •	1260*, 1270*, 1320,
Mayor, H.D.	820*		1410, 1560, 2270*
McCormick, G.E.	1940	Pagnes, P.	1950*
McG dre, C.D.	1540, 1550	Peacock, W.L.	1680, 1700
McQueen, J.L.	2340*	Peacock, W.L., Jr.	1710, 1720
Mellors, R.C.	250 *	Peeples, W.J.	1500*, 1510*
Melnick, J.L.	820	Peltier, A.	300*
Metzger, J.F.	590 830*	Peterson, W.C., Jr.	2970 *
Meysel, M.N. Mikhailov, I.F.	830* 260*, 530*, 1200*	Petuely, F.	1280*, 1520*
Mikhailova, Yu.M.	1150	Pishchurina, M.M.	810, 830
Miki, T.	2210	Pittman, B. Plexico, K.L.	1470
Mindlina, R.S.	1150	Poetschke, G.	2480, 2510
Mirolyubova, L.V.	1210*, 1220*	Pollack, V.E.	310*, 840* 2960, 2980*
Moller, H.F.	2940	Pomeres-Lebron, A.	550*, 1080, 1350,
Montague, T.S.	1330	2002011, 11.	1390
Montgomery, C.H.	1920*	Portnoy, J.	1960*
Moody, M.D.	460, 930, 990*,	Pushkova, K.T.	2040, 2770
	1050, 1060, 1460*,	•	
	1470*, 1480*, 1490*,	<u>R</u>	
Mr. Test and Mr. D	1500, 1510		
Mordvinova, N.B.	1150	Rantz, L.A.	1530
Morrissey, R.A.	1310	Rapp, F.	2120
Mounter, W.	2 90	Rauch, H.C.	1530*
Mueller, F.	2260* 1600	Ravaioli, L.	560 *
Mufson, M.A. Muschel, L.H.	1850, 2660	Redmond, D.L.	1020*
Mascher, D.H.	10,00, 2000	Reilly, H.W.	1910
<u>N</u>		Reimers, E. Rezaian, J.	1290*
"		Richards, O.W.	2960 320*
Nairm, R.C.	270*, 540*	Rifkind, D.	1610
Naito, M.	2190	Riggs, J.L.	2280*, 2290*
Nakamura, S.	2200	Roa, P.V.	570*
Naumann, H.H.	280*	Robinson, R.Q.	2380, 2420*
Nelson, J.D.	1030, 1040, 1230*,	Rodnan, G.P.	2870
	1240*, 2060	Roitt, I.M.	2850
Nicholson, L.R.	2020	Ross, M.R.	910*
Niel, G.	1860, 1870, 1930*,	Rozhanskiy, I.N.	1300*
	1970	Rudey, Z.Kh.	1120

<u>s</u>		$\underline{\mathtt{T}}$	
Sachs, M.V.	2810	Takahashi, M.	2210
Sadun, E.H.	2550, 2560, 2570,	Tamura, R.	290
	2600*, 2610*, 2620*,	Tanaka, N.	360 *
	2630*, 2640*, 2650*	Tararin, R.A.	630 ¥
Sanders, R.W.	1850	Tateno, I.	2190*, 2200*
Santini, R.	1800	Teague, P.	2840
Scarpa, B.	1760	Thayer, W.R.	2830
Schaeffer, M.	21+20	Thomas, B.	2510*
Schiller, E.L.	2580 1180	Thomason, B.M.	1080, 1330*, 1340*, 1350*, 1360*, 1370*
Schneider, H. Schneider, N.J.	2340		1380*, 1390*
Schuit, H.R.E.	2940	Thompson, G.R.	2990*
Scott, L.V.	2070, 2080	Tiedemann, J.	1720
Seegal, B.C.	730*, 740*	Tomov, A.	1400*
Sekino, K.	2130, 2240	Topleninova, K.A.	2350*
Sellers, T.F.	2530	Toyoshima, K.	2210*
Shaughnessy, H.J.	1310*	Tozzini, F.	640 x
Shepard, C.C.	2740, 2750, 2760	-	
Shevliagin, V.Ya.	330 *	<u>U</u>	
Shpagina, M.K.	1420		
Siboulet, A.	1970*	Uehleke, H.	310
Siegel, A.C.	1580	Updyke, E.L.	1480, 1490
Siegel, M.	3010	••	
Silverstein, A.M.	1980*	$\overline{\Lambda}$	
Simpson, W.G.	1740* 580*	Voicmon A	3,000*
Sinitskiy, A.A.	2400	Vaisman, A. Vanini, G.C.	1990* 2710
Slotnick, V.B. Smith, C.W.	590 *	Varga, D.T.	2510
Sonea, S.	2050*	Varquez, J.J.	2870
Sonnenwirth, A.C.	600 *	Veselov, V.A.	2040, 2770
Spasov, S.	1400	Vignali, C.	2000*, 2010*
Spielman, D.W.	1500, 1510	Vogel, R.A.	2520*, 2530*
Spink, W.W.	340*	Voller, A.	2720*
Spiro, H.M.	2830	Vozza, R.	920*, 2100*, 2430*
Sternberg, T.H.	2500*		
Stevens, R.W.	1790	<u>W</u>	
Streamer, C.W.	1540*, 1550*	**************************************	BEOV
Stulberg, C.S.	610*, 1000, 1010,	Waaler, E.	750* 2540*
	1100, 1260, 1270,	Walzer, R.A.	2330
	1320*, 1410, 1560,	Wamberg, K.	15 ⁴ 0, 1550
Cuciuma A	1570, 2270 2190	Wang, W.L.L. Warfield, M.A.	1560*, 1570*
Sugiura, A. Suhrland, S.	1920	Watkins, J.R.	890, 900
Sulkin, S.E.	890, 900, 1440	Weinert, A.	1160
Surfitz, 0.	1570	Weir, D.M.	2950, 3000*
Suzuki, S.	620*, 21.90, 2200	Werth, G.	200
Sylvana Chemical Co.	350 *	Whitaker, J.A.	950, 1030*, 1040*,
-			1230, 1240, 1410*,
			2060 *

White, J.D. 370*, 850* 380* White, R.G. 3010* Widelock, D. 2560, 2570, 2600 2610, 2620, 2650 Williams, J.S. 1540, 1550 Williams, P.M. 2360* 990, 1050*, 1060* 2110* Wilsnack, R.E. Winter, C.C. Witmer, R. 2860, 3050 Wood, R.M. 2530 870, 940, 2390 Woodward, P. Woodward, T.E.

 $\underline{\underline{Y}}$

Yarashus, D.A. 3060* Yarbrough, W.B. 2380

<u>Z</u>

Zavarzin, A.V. 630
Zelenkova, L. 1590*
Ziff, M. 1730
Zilisteanu, C. 1070
Zinovieva, I.S. 1420*

Zuelzer, W.W. 1410, 1560, 1570

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13 ABSTRACT				

This volume is one of a series of six annotated bibliographies on various aspects of immunofluorescence and its use. Citations cover the period 1905 through 1962. Volume V is divided into two major sections. The first section contains 25 annotated citations to review articles on immunofluorescence arranged according to major subject areas. The second section is devoted to diagnostic techniques. It contains 221 cross-references to citations in the other volumes of this series, arranged to correspond with subject matter areas in those volumes. A complete author index to the 306 citations is included.

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